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Cell-free translation in reversed micelles

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Cell-free translation in reversed micelles (RM) of surfactants in organic solvents is demonstrated using as an example the synthesis of human interleukin-2 by the wheat germ translation system solubilized in Brij 96 (oleyl-poly(10)oxyethylene ether) RM in cyclohexane. The translation system components and the product were recovered from the RM system by acetone precipitation. The recovery and translation reaction yields depended on the degree of surfactant hydration. The translation yields in Brij 96 RM were close to that observed in regular aqueous solution. The Brij 96 RM system is regarded as a promising media for the cell-free synthesis of hydrophobic proteins. Meanwhile, no translation reaction was observed in Aerosol OT (sodium bis(2-ethylhexyl) sulfosuccinate) RM in octane, which presumably is due to the ability of Aerosol OT to bind Mg^{2+} ions necessary for the functioning of the translation apparatus.

Cell-free translation; Interleukin; Micellar enzymology; Reversed micelle

1. INTRODUCTION

The phenomenon of enzyme catalysis in reversed micelles (RM), discovered in 1977 by Martinek et al. [1], is now under intensive study in dozens of laboratories (see for review [2–6]). The particular interest in this field is conditioned by the fact that RM systems provide unique possibilities for reconstructing the natural membrane microenvironment of biopolymers and their supramolecular assemblies. They permit the deliberate variation of a wide range of physico-chemical properties of this microenvironment, e.g. surfactant hydration, type of packing etc. which is of great importance for studying the membrane regulation of biochemical processes [6].

Although a lot of work on enzymes in RM systems has been done during the last decade, much less is known about the behavior of functional complexes of proteins and nucleic acids in this microheterogeneous medium. Luisi et al. [7] analyzed the structural changes in nucleic acids upon their incorporation into RM. The enzymatic processing of nucleic acids in RM systems, in particular the RNA hydrolysis with ribonuclease [8] and restriction of plasmids and λ DNA [9], have also been described. Besides these rare examples no systematic studies of the nucleoprotein complexes in RM have been performed until now.

It is also well known that many important biological processes involving nucleic acids proceed in the vicinity of, or directly in, biological membranes, and one could expect that the membrane physico-chemical state may strongly affect these processes. The protein translation on ribosomes evidently belongs to such examples [10].

In this paper we demonstrate the possibility of performing cell-free protein translation in RM using as an example human IL-2 synthesis by the wheat germ translation system, solubilized in Brij 96 RM in cyclohexane.

2. MATERIALS AND METHODS

The wheat germ translation system was prepared according to Roberts et al. [11]. The absorbance of the wheat germ extract (WGE) at 260 nm was equal to 80–100 optical units. Cell-free transcription of the mature form of human IL-2 was carried out according to Gurevich et al. [12].

The translation reaction in aqueous media was performed using a modified procedure [11]. Briefly, the translation reaction system (25 μl) containing 8 μl of WGE, 0.1 μg creatine phosphokinase (EC 2.7.3.2.; Sigma, 350 U/mg) and 0.5 μg of IL-2 mRNA in buffer B (40 mM HEPES, pH 7.6), 2 mM magnesium acetate, 112 mM potassium acetate, 4 mM DTT, 0.25 mM spermidine, 8 mM phosphocreatine, 25 μM each amino acid without Met (all from Sigma), 10 μCi [^{35}S]Met (Amersham, >1,000 Ci/mmol), 2 mM ATP, 50 μM GTP) was prepared

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Abbreviations: Aerosol OT, (sodium bis (2-ethylhexyl) sulfosuccinate); Brij 96, oleyl-poly(10)oxyethylene ether; DTT, dithiotreitol; IL-2, interleukin 2; HEPES, *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid); PAA, poly(acrylamide); RM, reversed micelle; TCA, trichloracetic acid; WGE, wheat germ extract.

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by mixing the corresponding components at 0°C immediately prior to the experiments described below. The reaction was started by increasing the temperature to 24°C after which it was run at 24°C for 40 min. The translation was stopped by adding 500 μl of 10% TCA to the reaction mixture. The precipitate formed was centrifuged at 8,000 rpm using an Eppendorf centrifuge for 10 min (0°C), washed (resuspended and precipitated again) with cold (0°C) acetone, dried, dissolved in the sample buffer for electrophoresis and analysed by 12% polyacrylamide (PAA) gel electrophoresis. The gels were stained with Coomassie blue and scanned using a Shimadzu CS-9000 densitrometer. The autoradiographic pictures of the gels were made using R-film. The gel lanes were cut and their total ^{35}S radioactivity was determined in a toluene scintillation solution using a Rackbeta β-counter.

The translation in RM was carried out using the following procedure. In a typical experiment 20–60 μl of buffer B were solubilized in 1 ml of 0.15 M Brij 96 (Sigma) in cyclohexane. The mixture obtained was shaken and incubated at room temperature until it became optically transparent (1–2 min). 25 μl of the translation system, prepared as described above, was incubated for 1 min at 24°C and then added to the micellar solution. The micellar solution was shaken again until it became optically transparent (1 min) and then incubated at 24°C for 40 min. The reaction was stopped by the addition of 1 ml of cold (0°C) acetone, and the system was centrifuged at 8,000 rpm as above in an Eppendorf centrifuge for 10 min (0°C). The precipitate was washed (resuspended and precipitated again) in 1 ml of cold acetone and 100 μl of 10% TCA, then 1 ml of cold acetone (2 times), dried, then dissolved in sample buffer for electrophoresis and analyzed as described above.

The cell-free translation in Aerosol OT RM was performed analogously to the described procedure. In this case 0.05–0.3 M Aerosol OT (Merck) solution was used for the preparation of the RM system. The translation products were precipitated from the reaction system and analyzed as for the Brij 96 RM system.

3. RESULTS AND DISCUSSION

The solubilization of the translation system in both Brij 96 and Aerosol OT RM results in the formation of an optically transparent solution, which provides evidence about formation under the studied conditions of microemulsions (water in oil type), containing the translation system components.

After 40 min incubation the translation system components and products were precipitated from the RM solution by adding acetone and analyzed by gel electrophoresis.

It should be taken into account that the extent of the protein recovery from water and RM system may differ significantly. Therefore the recovery efficacy was estimated from the gel electrophoresis data (Fig. 1A) by comparing the amounts of a protein corresponding to the aqueous solution and RM system (lanes 1 and 2–4). The total amount of the protein in each lane was determined using a scanner densitometer.

The data presented in Table I shows that the extent of the protein recovery from the RM system depends on the surfactant hydration degree. This dependence is presumably explained by the rise in protein solubility with the increase in water content in the cyclohexane–acetone–water mixture, as observed before in a number of other cases (unpublished data). In all the cases studied

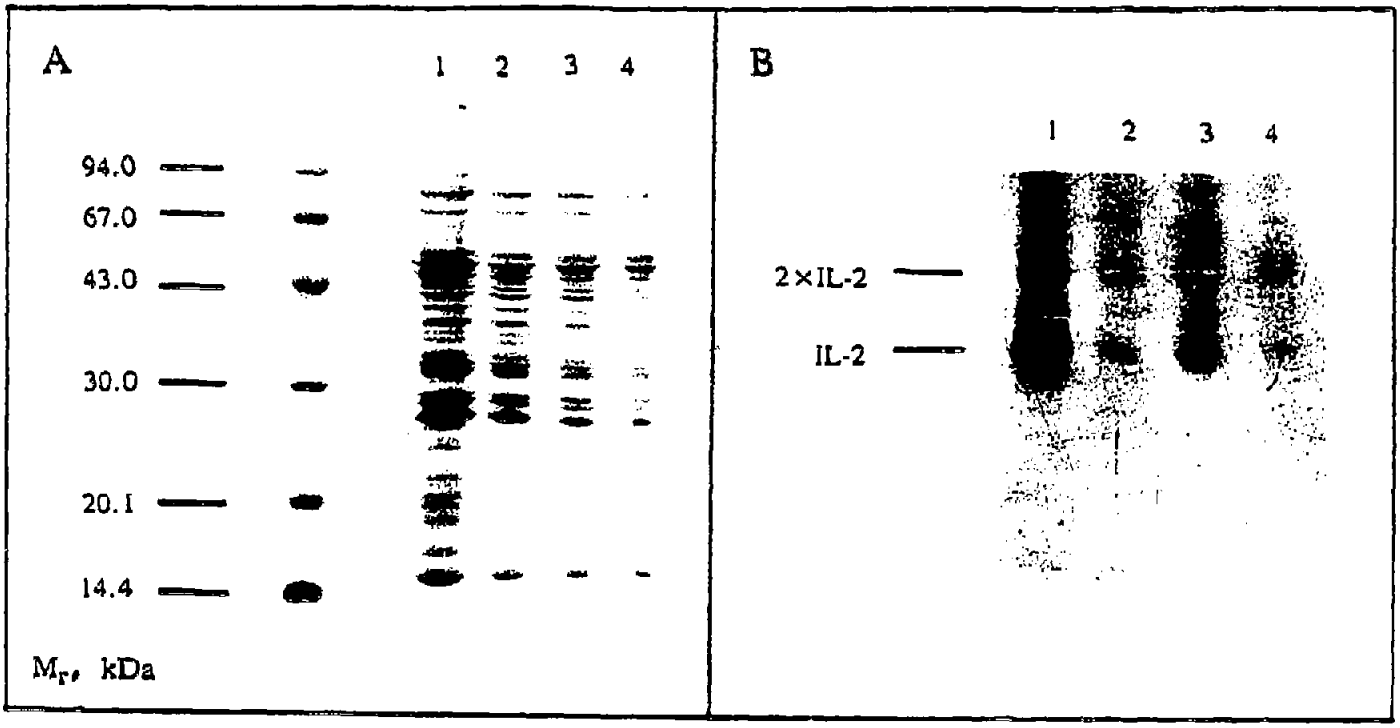

Fig. 1. (A) The gel electrophoresis (Coomassie blue staining) and (B) autoradiograph patterns of the translation system precipitates. Translation was performed in aqueous solution (lane 1) or Brij 96 RM in cyclohexane (lanes 2–4). The degree of surfactant hydration in the RM system is equal to 16.5 (lane 2), 24.0 (lane 3) or 31.5 (lane 4). The major bands, corresponding to the IL-2 monomer and dimer (2 × IL-2) are indicated by the arrows. (The amounts of radiolabelled protein observed in B depends both on the yield of protein synthesis and on the efficacy of the subsequent protein recovery from RM. The real yields of protein translation are presented in Fig. 2.)

Table I

Efficacy of a protein recovery and the translation yield[a]

Lane number	$[H_2O]/[Brij\ 96]$ (for the RM system)	Recovery yield (%)	^{35}S radioactivity of the lane (cpm)
1	in water	100	25,600 (± 450)
2	16.5	33	3,050 (± 150)
3	24.0	27	6,950 (± 250)
4	31.5	9	2,800 (± 100)

[a]The translation was performed either in aqueous solution or in Brij 96 RM and the protein was precipitated as described in Materials and Methods. The results of a typical experiment which was independently repeated several times are presented in Table I and Figs. 1 and 2.

the protein recovery from RM system (Table I) is less effective than that from aqueous solution.

Fig. 1B presents the autoradiograph of the gels shown in Fig. 1A. It can be clearly seen that IL-2 translation takes place both in aqueous media and in the Brij 96 RM system. The amount of the translation product detected in the case of RM depends on the degree of surfactant hydration (the molar ratio $[H_2O]/[Brij\ 96]$).

In order to estimate the translation reaction yield in the RM system the bands were cut from the gels and their ^{35}S radioactivity measured (Table I). The amount of protein synthesized in the RM system was determined as the ratio of the band radioactivity to the protein recovery efficacy at a given hydration degree.

As is shown in Fig. 2 the IL-2 translation yield in Brij 96 RM in cyclohexane steadily increases with an increase of in hydration degree. The reason for this phenomenon may be quite complicated and needs further study; however, the data obtained provides evidence that the cell-free translation in Brij 96 RM proceeds with yields comparable to that observed in aqueous solution.

The nature of the micelle-forming surfactant is an important factor influencing the translation in RM. In particular, we did not observe protein synthesis in the micellar system formed by an anionic surfactant (Aerosol OT in octane, Fig. 2). This may be due to the well-known ability of the Aerosol OT molecule to bind Mg^{2+} ions necessary for the translation reaction [13].

The RM systems are regarded as promising media for modelling the membrane microenvironment of biopolymers and their functional supramolecular complexes [5,6]. It is probable that they also may serve as good models for the study of membrane organization of the translation apparatus and, in particular, may become useful for the synthesis of hydrophobic membrane proteins.

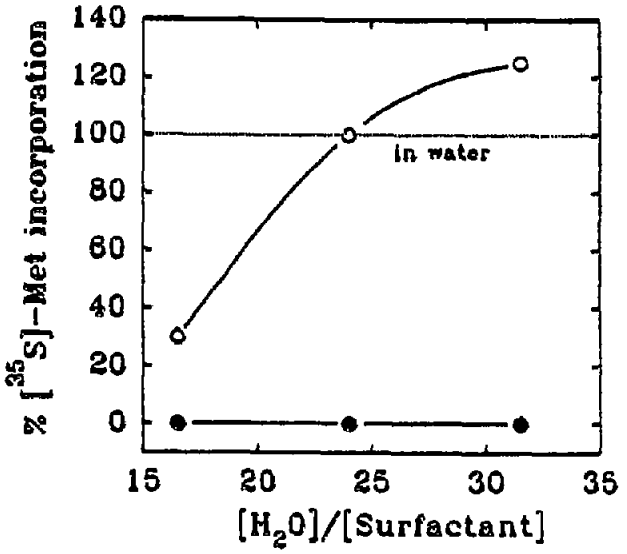

Fig. 2. The yield of cell-free translation in Brij 96 (○) and Aerosol OT (●) RM systems. The yield of the translation reaction observed in aqueous solution is shown by the dotted line.

REFERENCES

[1] Martinek, K., Levashov, A.V., Klyachko, N.L. and Berezin, I.V. (1977) Dokl. Acad. Nauk SSSR (Russ.) 236, 920-923.
[2] Pileni, M.P. (1989) Structure and Reactivity in Reversed Micelles, Elsevier, Amsterdam.
[3] Luisi, P.L. and Magid, L.J. (1986) CRC Crit. Rev. Biochem. 20, 409-474.
[4] Waks, M. (1986) Proteins 1, 4-15.
[5] Martinek, K., Klyachko, N.L., Kabanov, A.V., Khmelnitsky, Yu. L. and Levashov, A.V. (1989) Biochem. Biophys. Acta 981, 161-172.
[6] Klyachko, N.L., Levashov, A.V., Kabanov, A.V., Khmelnitsky, Yu. L. and Martinek, K. (1991) in: Kinetics and Catalysis in Microheterogeneous Systems (Gratzel, M. and Kalyansundaram, K. eds.) pp. 135-181, Marcel Dekker, New York.
[7] Imre, V.E. and Luisi, F.L. (1982) Biochem. Biophys. Res. Commun. 107, 538-545.
[8] Wolf, R. and Luisi, F.L. (1979) Biochem. Biophys. Res. Commun. 89, 209-217.
[9] Hanley, A.B., Furniss, C.S.M., Kwiatkowska, C.A. and Mackie, A.R. (1991) Biochem. Biophys. Acta 1074, 40-44.
[10] Ryasanov, A.G. and Spirin, A.S. (1988) Usp. Biologich. Khimii. (Russ.) 29, 3-43.
[11] Roberts, B.E. and Paterson, B.M. (1973) Proc. Natl. Acad. Sci. USA 70, 2330-2334.
[12] Gurevich, V.V., Pokrovskaya, I.D., Obukhova, T.A. and Zozulya, S.A. (1991) Anal. Biochem. 195, 207-213.
[13] Eicke, H.-F. and Kvita, P. (1984) in: Reversed Micelles (Luisi, P.L. and Straub, B.E. eds.) pp. 21-35, Plenum, New York.